Interaction of Ada Protein with DNA Examined by Fluorescence Anisotropy of the Protein†

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Received June 8, 1989; Revised Manuscript Received November 16, 1989

ABSTRACT: We made use of enhancement of fluorescence anisotropy of protein upon DNA binding to analyze interactions between Ada protein and DNA. Ada protein is a DNA repair enzyme that also acts as a transcription regulator. The isotropic fluorescence was not significantly affected upon interaction with DNA and could not be used as a signal for detection of the binding. The anisotropy did became larger because the binding to DNA reduces diffusion of the protein. The change was reproducible and independent of protein concentration and also independent of the degree of saturation of DNA with the protein when DNA was large; these values can readily be converted to the proportion of the complexed protein. The binding parameters were then determined by direct comparison between experimental and theoretical variations of anisotropy, with increasing concentrations of DNA. The theoretical variations were computed by considering the overlap of potential binding sites on the DNA lattice [McGhee & von Hippel (1974) *J. Mol. Biol. 86*, 469–489]. Binding does not seem to occur in a cooperative manner. The number of base pairs covered by a protein monomer was 7 ± 1; this number is independent of the salt concentration. The equilibrium association constant decreased from 4×10^7 to 3×10^5 M^{-1} for an increase of NaCl concentration from 0.1 to 0.2 M, thereby indicating the possible involvement of ionic interactions between phosphate groups of DNA and the protein.

Interactions between DNA and protein are essential in various biological phenomena, including replication, transcription, and DNA repair. A quantitative determination of thermodynamic parameters of these interactions would facilitate understanding of molecular mechanisms underlying these processes [for review, see von Hippel et al. (1975, 1984) and Lohman (1986)]. While various chemical and physical methods are now available for studies on DNA–protein interactions, spectroscopic measurements are pertinent as the binding equilibrium is not disturbed and the procedures are more economic of time (Bujalowski & Lohman, 1987). Measurements of intrinsic protein fluorescence have been made for this purpose (Kowalczykowski et al., 1981; Takahashi et al., 1983; Bujalowski & Lohman, 1987; Rajeswari et al., 1987). Various proteins contain tryptophan and/or tyrosine residues and hence are intrinsically fluorescent. This fluorescence can be readily measured even at low protein concentrations, and binding to DNA often, but not always, leads to changes.

We report here that formation of the DNA–protein complex can be demonstrated by measuring the anisotropy of fluorescence, even in the absence of a change in isotropic fluorescence. The anisotropy is related to the rotational movement of a macromolecule when the fluorophore is stiffly attached to the macromolecule (Cantor & Schimmel, 1980). Since formation of the DNA–protein complex significantly increases the molecular mass (which would decrease the rotational diffusion), an increase in the anisotropy value with complex formation would be expected. We used this approach to examine the thermodynamic characteristics of the nonspecific binding of the Ada protein to large DNA molecules.

Ada protein of *Escherichia coli* participates in the repair of DNA damaged by alkylating agents, such as *N*-methyl-*N*-nitrosourea and methylmethanesulfonate [for review, see Sekiguchi and Nakabeppu (1987) and Lindahl et al. (1988)]. These alkylating agents modify DNA in vivo and are highly toxic and mutagenic. The Ada protein has methyltransferase activities, which transfer the methyl groups from O^6-methylguanine and from methylphosphotriester to the cysteine residues of the protein (Lindahl et al., 1988; Takano et al., 1988; Sedgwick et al., 1988). The alkyl groups that are transferred are not further metabolized (Lindahl et al., 1988); thus, the Ada protein functions as a methyltransferase only once. However, the methylated form of Ada protein does act as a transcription regulator and promotes expression of the genes that are involved in the repair of alkylated DNA, including the *ada* gene itself (Sekiguchi & Nakabeppu, 1987; Sedgwick et al., 1988; Teo et al., 1986; Nakabeppu & Sekiguchi, 1986; Nakamura et al., 1988). This process, which is part of cellular defense mechanisms against DNA-damaging agents, is known as adaptive response.

In vitro studies revealed that the Ada protein binds to a specific region near the promoter of the *ada* gene and enhances the binding of RNA polymerase to the promotor (Nakamura et al., 1988; Sakumi & Sekiguchi, 1989). Examination of thermodynamic properties of DNA–Ada protein interactions should provide further insight in this action, as well as mechanisms related to DNA repair. As determination of the interaction of Ada protein with nonspecific DNA is a prerequisite for further analyses of any specific interaction (Takahashi et al., 1983, 1989) and will lead to a better understanding of the biological functions, a quantitative analysis of this interaction was made.

Materials and Methods

Protein and DNA. Ada protein was prepared as described by Nakabeppu et al. (1985), and the concentration was de-

† This work was supported by the Monbusho International Scientific Research Program. M.T. has been supported by grants from the exchange program between the Centre National de la Recherche Scientifique and the Japan Society for Promotion of Science.

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0006-2960/90/0429-3431$02.50/0

termined spectroscopically by using $\epsilon_{280} = 3.8 \times 10^4$ M^{-1} cm^{-1} (Nakabeppu & Sekiguchi, 1986). Highly polymerized calf thymus DNA (Sigma type I) was generally used as the nonspecific DNA. About 10 mg of dried calf thymus DNA was soaked for 2 days in 10 mL of buffer containing 20 mM Tris-HCl and 1 mM EDTA, pH 7.6 at 4 °C, and then dissolved by gentle agitation. Electrophoresis on agarose gel showed that this DNA solution was mainly constituted of fragments larger than 20K base pairs and that fragments smaller than 500 base pairs were absent. For some experiments, we used the 2686 base pair, 98 base pair, and 22 base pair DNA fragments without a specific binding site for Ada protein. The 2686 base pair fragment was prepared from digestion of the plasmid pUC19 by *EcoRI* enzyme, 98 base pairs from plasmid pRW574, as described by Hillen et al. (1981). The 22 base pair fragment was synthesized by an automatic DNA synthesizer (Applied Biosystem). The sequence was

5' ATGTGAGTTAGCTCACTCATTA
TACACTCAATCGAGTGAGTAAT 5'

Methylated DNA was prepared by treatment of calf thymus DNA by *N*-methyl-*N*-nitrosourea, as described by Nakabeppu and Sekiguchi (1986), and degraded to fragments of about 50 base pairs. All DNA concentrations are expressed in base pairs.

The experiments were done at 26 °C in a buffer containing 20 mM Tris-HCl, pH 7.5, 1 mM EDTA, and 1 mM dithiothreitol supplemented with the required concentrations of NaCl. When the anion effect was examined, Cl^- ions were replaced by other anions. All reagents were of analytical grade, and the buffers were passed through nitrocellulose filters with pores of 0.22 μm (Millipore, type GS) and kept in glassware. Use of plasticware was avoided to minimize the contamination of fluorescence.

Fluorescence Measurements. Isotropic (I_{55}) fluorescence and anisotropic fluorescence of Ada protein were measured at 342 nm (with Schott interference filters) upon selective excitation of tryptophan residues at 295 nm (with a bandwidth of 4 nm) in an SLM 8000 spectrofluorometer. The spectrofluorometer was equipped with two photodetectors to simultaneously determine horizontal and vertical polarized components of emission light. A home-built device ensures automatic rotation of the excitation polarizer. For each fluorescence anisotropy determination, 10 measurements of 10 s each were made and averaged. The average scatter of measurements was usually less than 0.003 anisotropy unit. Isotropic fluorescence intensity was determined on the same sample, using a vertically oriented polarizer in the incident beam and a second polarizer oriented at an angle of 54.7° to the former in the emerging beam. Fluorescence signals from contaminants in buffer or DNA sample were usually less than 1% of the total fluorescence and, hence, negligible under these conditions. Nevertheless, the contaminant signal was systematically subtracted for both isotropic and anisotropic measurements.

Analysis of Binding Data. The binding parameters (intrinsic association constant K, cooperativity ω, and site size n) were determined by direct comparison between theoretical variation of the anisotropy value and data obtained in experiments. For each set of protein and DNA concentrations, the amount of bound protein was computed with a set of DNA binding parameters by resolving the equation of McGhee and von Hippel (1974) and using the half-interval method described by Takahashi (1987, 1989) and Takahashi et al. (1989). The theoretical anisotropy value r was computed by using

$$r = r_1F_1 + r_2F_2 \quad (1)$$

where r_1 and r_2 refer to anisotropies of the free and bound proteins, respectively, and F_1 and F_2 refer to the fraction of the total isotropic fluorescence due to each form of protein. The anisotropies were defined as $r = (I_\parallel - I_\perp)/(I_\parallel + 2I_\perp)$. $I_\parallel$ and $I_\perp$ were, respectively, the parallel and perpendicular components of the fluorescence.

A series of theoretical curves was generated by systematically varying the binding parameters. The quality of the fit to the experimental data was evaluated by visual inspection and by means of a residual

$$S^2 = \sum(r_{theor} - r_{exp})^2$$

where r_{theor} and r_{exp} were the theoretical and experimental anisotropy values, respectively.

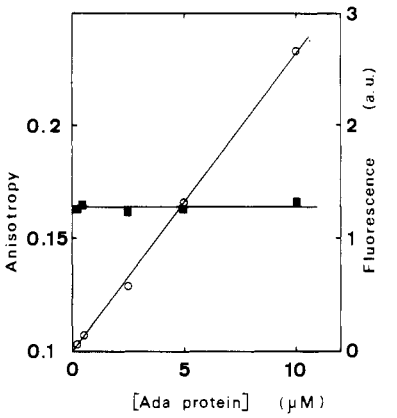

FIGURE 1: Concentration independence of the fluorescence anisotropy of the Ada protein. Isotropic fluorescence (○) and fluorescence anisotropy (■) of Ada protein in various concentrations were measured in 20 mM Tris-HCl and 100 mM NaCl, pH 7.5 at 26 °C, as described in the text.

Results

Independence of Free Ada Protein Concentrations in Anisotropy. Isotropic fluorescence and anisotropic fluorescence of Ada protein were measured at protein concentrations varying from 0.2 to 10 μM in the presence of 100 mM NaCl. Isotropic fluorescence increased linearly with increasing protein concentrations, while the anisotropy value (r) was independent of the concentration and 0.165 ± 0.003 (Figure 1). This shows that the protein exists in only one quaternary structure form at any protein concentration and thus the absence of association or dissociation of protein subunits. Therefore, one can attribute only one r value to free protein, and the conversion of anisotropy value to the proportion of complexed protein can be made by eq 1. Anisotropy values of the protein fluorescence were also independent of the concentration of the salt.

Changes in Anisotropy of Protein Fluorescence upon DNA Binding. Under the same conditions, the anisotropy value of protein fluorescence was progressively increased with successive additions of DNA to the protein solution (Figure 2), and reached a plateau. The change in anisotropy occurred immediately after DNA was added and remained unchanged for at least 15 min. The maximum anisotropy value obtained was about 0.2 and was independent of protein concentrations from 0.2 to 2 μM (Figure 2), though a larger amount of DNA was

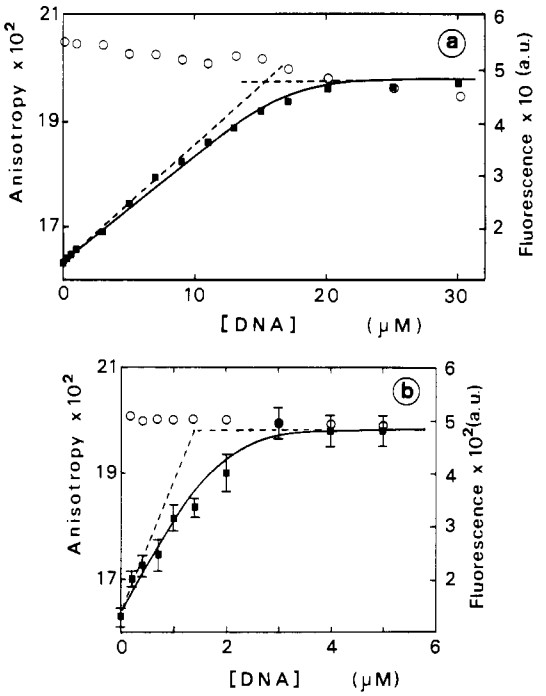

FIGURE 2: Determination of binding parameters of Ada protein–DNA interaction by examining variations of fluorescence anisotropy with DNA concentrations. DNA was added successively to 2 (panel a) and 0.2 μM (panel b) Ada protein solution under the same conditions described for Figure 1. After DNA was added, the isotropic fluorescence (○) and anisotropy (■) were recorded. The isotropic fluorescences were not corrected for the inner filter effect from the absorption of DNA. The average scatters of anisotropy measurements are presented by error bar in (b) because they were significantly larger than those seen with higher concentrations of protein (a). The broken line in (a) was drawn to estimate the site size and is reproduced in (b). The theoretical curve (—) superimposed on the anisotropy signals was obtained with $K = 4 \times 10^7\ M^{-1}$, $\omega = 1$, and $n = 7$.

required for higher concentrations of the protein to reach a plateau.

In contrast to the anisotropy, the intensity of isotropic fluorescence was little modified by added DNA (Figure 2). The slight decrease in intensity did not relate to changes in anisotropy and could be explained by the inner filter effect due to the slight absorption of DNA at 295 nm. In the presence of a low concentration of protein the maximum anisotropy was achieved with low concentrations of DNA; the decrease of isotropic fluorescence was negligible even in the presence of DNA in a sufficient amount to reach the maximum anisotropy (Figure 2b). Change in the isotropic fluorescence was thus related to the concentration of DNA and not to the complex formation. This further implies that the slight decrease in fluorescence intensity induced by the addition of DNA is due to an inner filter effect. The fluorescence intensity was not significantly modified with the DNA binding. In this case, the molar fraction of the complexed protein (f_2) is presented as a fraction of the isotropic fluorescence F_2 (Lakowicz, 1983) and is expressed by

$$f_2 = (r - r_1)/(r_2 - r_1) \qquad (2)$$

where f_2 = [complexed protein]/[total protein] and r_1, r_2, and r are anisotropy values of free and complexed protein and the mixture, respectively. The amount of bound protein is proportional to the change of anisotropy.

Figure 2a shows that, in the presence of 2 μM of Ada protein, the amount of complexed protein increased first linearly with increasing amounts of added DNA; hence, there seems to be a rather tight binding of the protein to DNA. The

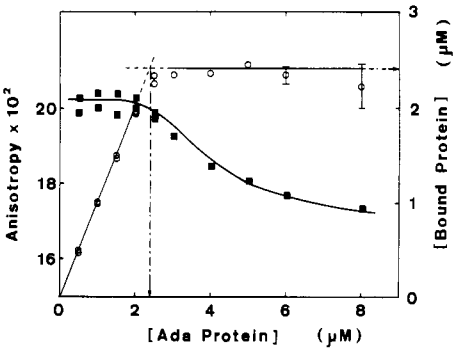

FIGURE 3: Determination of the site size using the titration of DNA by Ada protein. Ada protein was added successively to 20 μM DNA, under the conditions described in Figure 2, and fluorescence anisotropy was measured after each addition. The fluorescence anisotropy (■) was converted to the amount of bound protein (○) by using eq 2 and $r_2 = 0.205$.

crossover of the extrapolated initial slope and the plateau value gave the site size of 7–8 base pairs/Ada protein monomer. Because of the formation of aggregates, we could not perform experiments at lower salt concentrations where the binding is usually favored (Record et al., 1976) and the titration easier. Therefore, an inverse titration, i.e., the addition of protein to the DNA solution, was performed to confirm the binding stoichiometry (Figure 3).

At the beginning of titration, a point at which the protein/DNA ratio is low, the anisotropy of fluorescence was large (Figure 3), presumably because all the protein molecules were bound to DNA. The value was about 0.2 and corresponded to the maximum anisotropy value observed by the titration of protein by DNA. An increase in protein concentration to 2 μM for 20 μM base pairs of DNA did not alter the anisotropy value, probably reflecting the fact that there was a sufficient number of DNA molecules to bind almost all the protein molecules. When the protein concentration exceeded 2 μM, there was a decrease in the anisotropy, indicating the presence of free protein molecules. At such DNA/protein ratios, there was not a sufficient number of DNA molecules to bind all of the proteins. The absence of variation of anisotropy with changes of protein/DNA ratio from 0.025 to 0.1 indicates that the fluorescence anisotropy of the complexed protein is independent of the degree of saturation of DNA by the protein. There is only one anisotropy value for the bound protein, which supports eq 2.

Using this equation, we converted the anisotropy value to the amount of bound proteins. From the crossover of the slope and the plateau we noted that 20 μM base pairs of DNA is saturated with 2.4 μM protein (Figure 3). The amount of saturating protein determined at the plateau value was also 2.4 μM for 20 μM base pairs of DNA (Figure 3). Thus, the stoichiometry is about 8 base pairs/monomer, a figure close to the 7.5 base pairs determined by the titration of protein by DNA.

Effect of Protein Concentration. Although the anisotropy value obtained with saturating amounts of DNA was independent of the protein concentration, the binding curve did depend on the concentration (Figure 2). A larger DNA/protein ratio was required to saturate the protein when the concentration was lower (cf. parts a and b of Figure 2). The curvature is also more pronounced with 0.2 μM than with 2 μM Ada protein. This indicates that a protein concentration of 0.2 μM is not large enough to neglect the presence of free protein. Under these conditions, the association constant can be determined by analyzing the binding curve.

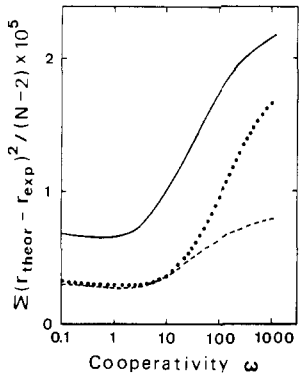

FIGURE 4: Determination of cooperativity by error analysis. To estimate the cooperativity ω of DNA–Ada protein interaction observed in Figure 3, minimum $\sum(r_{theor} - r_{exp})^2/(N-2)$ was determined for various ω values by optimizing K value at $n = 7$ and is presented as a function of ω (—). N is for the number of experimental points. The same analysis was performed on results obtained with 2 μM Ada protein at 0.15 (---) and at 0.18 M (···) NaCl.

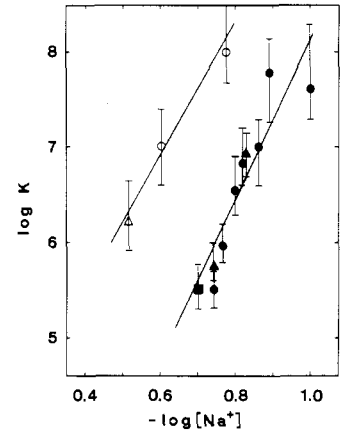

FIGURE 5: Salt dependence of Ada protein–DNA interaction. Variation of the association constant K with varying salt concentrations is presented in double-log plot. K values were determined as described in the text by a least-squares method for various salt concentrations with 0.5 (○, ●), 2 (△, ▲), and 5 μM (■) Ada protein. K values in Cl^- buffer are presented by solid symbols and in F^- buffer by open symbols. When K values were outside the error bars, the S^2 value was significantly larger (>50%) than the minimum S^2.

The analysis was made by comparison of theoretical and experimental variations of anisotropy. The site size $n = 7$ and 8 base pairs was used, and K and ω were systematically varied. The best fit was obtained with a cooperativity value close to 1 (Figure 4). The presence of cooperativity was not significant because S^2 obtained with $\omega = 1$ was not significantly different (<5%) from the minimum S^2 (Figure 4). The fit was better with a site size of 7 base pairs than with 8 base pairs. The intrinsic association constant K was 4×10^7 M^{-1} when the binding was not cooperative and $n = 7$. The theoretical variation of anisotropy, computed with these binding parameters for higher protein concentration (2 μM), also showed a good fit with the experimental data (Figure 2a). Thus, these values were consistent with results obtained by using a 10 times higher concentration of protein.

Salt Effect. Since DNA–protein interactions usually depend on cation concentrations due to a competition between cation and protein for the binding to DNA (Record et al., 1976), the salt effect was studied to estimate the situation in vivo and to characterize the interaction. At higher salt concentrations, larger amounts of DNA were required to reach a plateau value of anisotropy, suggesting lower binding affinity. The plateau value, r_2, also decreased slightly from 0.2 to 0.19 with increase in salt concentrations from 0.1 to 0.2 M.

A series of theoretical curves were generated by varying ω as well as K and n. At given n and ω values, K was determined by minimizing S^2. The minimum S^2 values at various ω values were compared to optimize the ω value. The operation was then repeated for another n value. The best fits were obtained with a very small ω, in all cases, $\omega = 1$ at 0.15 M NaCl and 2 at 0.18 M NaCl (Figure 4). The S^2 values obtained with $\omega = 1$ were not significantly different from the minimum S^2 obtained at best fits. Thus, the binding showed no significant cooperativity from 0.1 to 0.2 M NaCl.

The site size n also appeared to be independent of salt concentrations and was about 7 base pairs. In the course of the fitting, n was varied from 5 to 10. The best fits were achieved, in all cases, with $n = 7 \pm 1$. At this stage, the association constant K, was redetermined by using $\omega = 1$ and $n = 7$. Since it is also possible to modify the final anisotropy value (r_2) in this process, we examined the influence of the uncertainty on the r_2 value on the determination of K. We determined K by minimizing S^2 at various r_2 and then compared the S^2 values to estimate r_2. The best fits, i.e., minimum S^2, were obtained with r_2, as estimated from the plateau or close to it (±0.002).

K thus determined was also independent of the protein concentration, at different salt concentrations (Figure 5). In contrast, K depended upon the concentration of salt, and K decreased from 4×10^7 to 3×10^5 M^{-1} with increasing salt concentration, from 0.1 to 0.2 M. A double-log plot of the variation of K with salt concentration is shown in Figure 5. This approach was proposed by Record et al. (1976) to estimate the number of ion pairs formed between protein and DNA. The plot was linear with a slope $m'\psi$ of about 9 (±2). Since $\psi = 0.88$ for double-stranded DNA, this corresponds to the formation of $m' = 10 \pm 2$ ion pairs per binding of one Ada protein monomer.

The figure was large compared to the site size of 7 base pairs. This indicates the release of anions from protein upon DNA binding (Record et al., 1977). We therefore did an experiment using F^- ions instead of Cl^- ions. F^- ions bind to protein with lower efficiency than Cl^- ions and thus interfere much less with the interaction of protein with DNA (Kowalczykowski et al., 1981). In fact, the binding affinity was improved in the buffer with F^- ions compared to that in the buffer with Cl^- ions at a corresponding salt concentration (Figure 5). The replacement of Cl^- by F^- did not modify the anisotropy of free or bound protein. The site size was about 7 base pairs. There would not be a drastic structure change of the protein or of the complex upon the replacement of Cl^- by F^-. Thus, the improvement in binding affinity would rely on the release of anions from the protein. There was little difference in the slope (7 ± 1) in the double-log plot; i.e., the number of anions released from the protein was probably less than 2.

Effect of Length and Methylation of DNA. To obtain information on the structure, the effect of the length of DNA on anisotropy of the complexed protein was examined at various protein/DNA ratios. With a rather large 2686 base pair fragment, the anisotropy value was independent of saturation and similar to the value obtained with calf thymus DNA (Figure 6). With the 98 base pair fragment, the anisotropy value was slightly smaller at the ratio of 1 Ada/fragment (Figure 6), but at a higher protein/DNA ratio, the value was as large as that seen with the larger DNA. With very small DNA (22 base pair oligonucleotide), the anisotropy value was much smaller at a low protein/DNA ratio. The value progressively increased with increase in the protein/DNA ratio

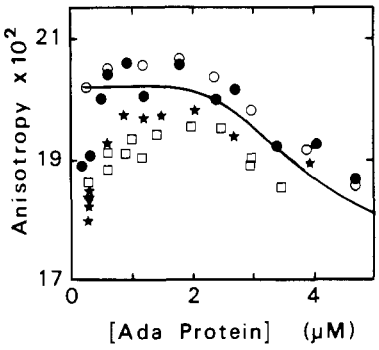

FIGURE 6: Effect of length and methylation of DNA. As for Figure 3, Ada protein was added successively to different types of DNA (20 μM in base pairs), and the fluorescence anisotropy was measured: (○) 2686 base pairs; (●) 98 base pairs; (□) 22 base pair fragment; (★) methylated DNA. The symbol ▼ represents the result with 20 μM methylated DNA and 20 μM unmodified calf thymus DNA. The results with calf thymus DNA (Figure 3) are indicated by continuous line (—).

but remained smaller than the value with a larger DNA (Figure 6). Thus, the anisotropy value clearly related to the length of DNA to which the Ada protein bound.

The binding to methylated DNA was also examined. The anisotropy was only slightly increased at a low protein/DNA ratio and progressively increased with increase in the protein/DNA ratio (Figure 6) simply because methylated DNA was small. The addition of nonmethylated large calf thymus DNA did not significantly increase the anisotropy (Figure 6). This shows that more than 90% of the protein bound to methylated DNA, even in the presence of equal amounts of nonmethylated DNA. Therefore, the binding affinity to methylated DNA should be, at least, 1 order higher than to unmodified DNA. Since the methylation of DNA is incomplete, the difference must be even larger. Further quantitative analyses were not feasible because of a variation in anisotropy with the degree of saturation and also because the methyl group can be removed from the methylated DNA by the methyltransferase activity of the Ada protein.

Discussion

We showed that fluorescence anisotropy of proteins can be used as a valid signal for the formation of DNA–protein complexes, and we analyzed interactions between Ada protein and nonspecific DNA using this approach. In the case of Ada protein, anisotropy measurements proved useful even though the change in isotropic fluorescence upon DNA binding is absent or weak. Anisotropy can be modified by a simple increase in molecular mass upon complex formation, with no change in the environment of the fluorescent residue (tryptophan and tyrosine), an event usually required for change in isotropic fluorescence.

The validity of the method was shown by the intrinsic association constant K and site size n determined for one protein concentration, a finding consistent with results obtained with a different protein concentration. The site size n determined by different titrations (the titration of protein by DNA, DNA by protein, and fitting analysis with a low protein concentration) was the same. The uncertainty concerning the anisotropy value was usually less than 0.003 unit, smaller than 10% of the value (0.03) observed for the change upon DNA binding. This order of uncertainty in determination of the concentration of the complex is acceptable and is frequently present in various spectroscopic measurements, including isotropic fluorescence (Kowalczykowski et al., 1981; Takahashi et al., 1983; Rajeswari et al., 1987).

Fluorescence anisotropy also provides structural information (Cantor & Schimmel, 1980; Kneal & Wijnaendts van Resandt, 1985; Bulsink et al., 1986). The anisotropy of free Ada protein is rather large and suggests low internal flexibility of the tryptophan residues. Independence of the anisotropy value from the protein concentration is consistent with the observation that Ada proteins remain in the monomer form in a wide range of concentrations (Bhattacharyya et al., 1988). Since isotropic fluorescence is not modified upon DNA binding, presumably because of the absence of any direct interaction between tryptophan residues and DNA, the enhancement of anisotropy is expected to relate to the decrease of global movement of protein. The addition of DNA mass to the protein probably produces this effect. In fact, the enhancement of anisotropy was smaller when the DNA fragment was very small and the protein/fragment ratio was lower than 1. With such small DNA, the anisotropy became larger with increase of protein/DNA ratio because the binding of additional protein increases the mass of complex and further reduces the diffusion. These observations exclude the dimerization of Ada protein on DNA and are consistent with the absence of cooperativity in DNA binding, concluded from analysis of binding equilibrium. The Ada protein, thus, binds to nonspecific DNA in monomer form, as expected for binding to the specific site (Sakumi & Sekiguchi, 1989).

The thermodynamic characteristics of Ada protein–DNA interaction were determined by comparison of theoretical and experimental anisotropy values instead of by comparison of the concentrations of bound protein that can be deduced from anisotropy values. This direct comparison would be more appropriate than fitting to values deduced from experimental data because the transformation of data could increase or distort the uncertainty. For example, the estimation of maximum signal change (here $r_2 - r_1$), which is required for the transformation of signal to the concentration of bound protein, is usually difficult, and misestimation leads to a distortion of data. This was avoided in our process by including r_2 as one of the fitting parameters.

The binding of Ada protein was not significantly cooperative. This is expected for protein which specifically binds to an isolated site near a promotor or to an alkylated DNA base, though some transcription regulation proteins (CRP, LexA) do bind to nonspecific DNA in a cooperative manner (Takahashi et al., 1979; Schnarr & Daune, 1984). The site size was about 7 base pairs. A similar number of base pairs (8 base pairs) was also required in the case of specific binding (Nakamura et al., 1988; Sakumi & Sekiguchi, 1989). This suggests that the binding mode of Ada protein to nonspecific DNA and that to specific DNA may be similar.

The nonspecific binding of Ada protein to DNA is most sensitive to salt concentration and depends on the nature of the anions, indicating a large contribution of electrostatic interaction between DNA and Ada protein. This is a typical characteristic of nonspecific (sequence-independent) binding. The number of cations released by one Ada protein monomer would be 8 ± 1 even if two anions are released. This number appears large compared to the site size of 7 base pairs, though not incompatible.

Acknowledgments

We are grateful to Dr. Guy Duportail and Alain Diab (Faculté de Pharmacie, Université Louis Pasteur, Strasbourg) for helpful advice and technical assistance with the fluorescence anisotropy measurements, to Drs. Elisabeth Bertrand and Robert Fuchs for stimulating discussions and continuous encouragement, to Dr. Patrice Koehl for improvement of the

manuscript, and to Liliane Diebolt for excellent secretarial assistance.

Registry No. 22 base pair fragment, 125685-20-3.

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Cisplatin Resistance and Mechanism in a Viral Test System: SV40 Isolates That Resist Inhibition by the Antitumor Drug Have Lost Regulatory DNA†

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Received July 10, 1989; Revised Manuscript Received October 27, 1989

ABSTRACT: Isolates of SV40 that have enhanced ability to survive inhibition by the antitumor drug cisplatin were selected by serial drug challenge in vivo. These mutant viruses have acquired specific deletions within the repeated regulatory motif $(GGGCGG)_6$ or GC box. This DNA element was shown previously to be a strong target of drug attack by cisplatin and other anticancer drugs in vitro and is an important viral and cellular DNA control sequence. Thus, drug resistance in this viral test system is dependent on the loss of important target DNA sequences. The results also indicate that drug efficacy may be related to the ability of certain anticancer drugs to attack regulatory DNA sequences containing strings of guanosines.

Simple platinum compounds such as *cis*-diamminedichloroplatinum(II) (cisplatin) and its derivatives have the ability to selectively inhibit the growth of certain tumor cells [see Nicolini (1988) for a review]. Originally identified as an agent that inhibited *Escherichia coli* cell division (Rosenberg et al., 1965), cisplatin has become one of the most widely used anticancer drugs. The primary functional target of cisplatin attack is believed to be DNA, and the inhibition of DNA synthesis is generally thought to be the process most deleterious to the viability of the tumor cell [reviewed by Sherman and Lippard (1987)]. Recently, however, the central

† This research was supported by a grant from the American Cancer Society and a seed grant from the California Institute for Cancer Research.

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0006-2960/90/0429-3436$02.50/0